

**WEST**[Generate Collection](#)[Print](#)**Search Results - Record(s) 1 through 2 of 2 returned.**☐ 1. Document ID: US 5432272 A

L1: Entry 1 of 2

File: USPT

Jul 11, 1995

US-PAT-NO: 5432272

DOCUMENT-IDENTIFIER: US 5432272 A

TITLE: Method for incorporating into a DNA or RNA oligonucleotide using nucleotides bearing heterocyclic bases

DATE-ISSUED: July 11, 1995

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Benner; Steven A.	CH-8006 Zurich			CH

US-CL-CURRENT: 536/25.3; 435/91.1, 435/91.41, 435/91.51, 536/25.33

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc
Image												

☐ 2. Document ID: US 5432272 A

L1: Entry 2 of 2

File: DWPI

Jul 11, 1995

DERWENT-ACC-NO: 1995-254483

DERWENT-WEEK: 200105

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TITLE: Incorporation of nucleotide analogues into oligo:nucleotide chains - by polymerase chain reaction on template contg. nucleotide analogue

INVENTOR: BENNER, S A

## PATENT-ASSIGNEE:

ASSIGNEE	CODE
BENNER S A	BENNI

PRIORITY-DATA: 1990US-0594290 (October 9, 1990)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5432272 A	July 11, 1995		013	C07H023/00

## APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 5432272A	October 9, 1990	1990US-0594290	

INT-CL (IPC): C07 H 23/00; C12 P 19/34

RELATED-ACC-NO: 1999-589901;2000-096152 ;2000-269883 ;2001-040322

ABSTRACTED-PUB-NO: US 5432272A

BASIC-ABSTRACT:

A method is claimed for incorporating at least one nucleotide unit bearing a heterocyclic base selected from the formulae shown in the figure into a DNA or RNA oligonucleotide chain, where R is the point of attachment of the base to position. 1 or a ribose or deoxyribose ring; X = N or CZ; Z = H or lower alkyl opt. substd. by NH<sub>2</sub>, COOH, OH, SH, aryl, indolyl or imidazolyl; Y = N or CH; and each ring contains no more than three consecutive N atoms. The method comprises: (a) synthesising an oligonucleotide template contg. at least one nucleotide unit as above; (b) dissolving the template in a buffered aq. soln.; (c) adding nucleoside triphosphates complementary to the nucleotide units in the template; (d) adding a DNA or RNA polymerase; and (e) incubating the mixt. to produce an oligonucleotide chain that is complementary to the template and contains at least one nucleotide unit as above.

USE - It is implied that the prods. may be useful for preparing labelled or immobilised nucleic acid probes, in the design of catalytically active RNA mols. (e.g. for antiviral or agricultural applications). in studies of nucleic acid structure, and for transcription into proteins contg. non-natural amino acids.

CHOSEN-DRAWING: Dwg.0/5

TITLE-TERMS: INCORPORATE NUCLEOTIDE ANALOGUE OLIGO NUCLEOTIDE CHAIN POLYMERASE CHAIN  
REACT TEMPLATE CONTAIN NUCLEOTIDE ANALOGUE

DERWENT-CLASS: B04 C06 D16

CPI-CODES: B04-B03C; C04-B03C; B04-E01; C04-E01; D05-H12D; D05-H18; D05-H18B;

CHEMICAL-CODES:

Chemical Indexing M1 \*01\*

Fragmentation Code

M423 M720 M903 N134 N153 Q233 V753

SECONDARY-ACC-NO:

CPI Secondary Accession Numbers: C1995-116346

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc
Clip Img	Image											

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Term	Documents
"5432272".DWPI,USPT.	5
5432272S	0
"5432272".PN..USPT,DWPI.	2
(5432272.PN.).USPT,DWPI.	2

**Display Format:**

[Previous Page](#)

[Next Page](#)

# WEST Search History

DATE: Tuesday, March 18, 2003

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
side by side			result set
<i>DB=USPT,DWPI; PLUR=YES; OP=ADJ</i>			
L1	5432272.pn.	2	L1
L2	Nanogold	11	L2
L3	Nanoprobe	77	L3
L4	5472881.pn.	2	L4
L5	Sperling-J\$.in.	15	L5
L6	Medalia-O\$.in.	1	L6
L7	nucleotide same sugar same phosphodiester same linkage same prymidine same terminal thiol	0	L7
L8	nucleotide and sugar and phosphodiester and linkage and (pyrimidine or purine) and terminal thiol	6	L8
L9	pyrimidine thiol group	0	L9
END OF SEARCH HISTORY			

Stabilization of double-stranded oligonucleotides using  
backbone-linked disulfide bridges.

AUTHOR: Gao H; Yang M; Cook A F  
CORPORATE SOURCE: PharmaGenics Inc., Allendale, NJ 07401.  
SOURCE: NUCLEIC ACIDS RESEARCH, (1995 Jan 25) 23 (2) 285-92.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199503  
ENTRY DATE: Entered STN: 19950404  
Last Updated on STN: 19950404  
Entered Medline: 19950321

AB A convenient, practical route to the synthesis of disulfide-bridged oligonucleotides has been developed. Aliphatic linkers with **terminal thiol** groups have been attached to the phosphodiester backbones of partially or fully complementary oligonucleotide sequences and oxidized to yield covalently closed oligonucleotides with disulfide bridges. This procedure has been used to prepare a duplex with disulfide bridges at both ends and stem-loop sequences with single disulfide bridges. Oxidation of a self-complementary duplex possessing **terminal thiol** groups produced both hairpin and duplex structures with disulfide bridges, the relative proportions of each being dependent upon the reaction conditions. These bridged hairpin and duplex structures were shown to be interconvertible by reduction and re-oxidation. The melting profiles of disulfide-bridged oligonucleotides were compared with the same sequences without bridges and with sequences possessing triethylene glycol bridges, and in all cases the introduction of disulfide bridges resulted in a considerable increase in thermal stability. EcoRI endonuclease was capable of cleaving a disulfide-bridged duplex possessing a recognition site for this enzyme, thus supporting a lack of distortion of the recognition site. The disulfide bridges could be cleaved using a large excess of DTT to regenerate the corresponding sulfhydryl compounds. A study of the serum stabilities of disulfide-bridged oligonucleotides showed that the bridged duplexes were much more stable than their unmodified counterparts, whereas the rate of degradation of the stem-loop structures was more dependent upon the size of the loop than the presence or absence of the disulfide bridge. In summary, we have described a novel methodology, employing commercially available reagents, for the stabilization of oligonucleotide duplexes or stem-loop structures by disulfide bridge formation.

89057464 PubMed ID: 3194200

TITLE: An oligodeoxynucleotide affinity column for the isolation of sequence specific DNA binding proteins.  
AUTHOR: Blanks R; McLaughlin L W  
CORPORATE SOURCE: Department of Chemistry, Boston College, Chestnut Hill, MA 02167.  
SOURCE: NUCLEIC ACIDS RESEARCH, (1988 Nov 11) 16 (21) 10283-99.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198901  
ENTRY DATE: Entered STN: 19900308  
Last Updated on STN: 19900308  
Entered Medline: 19890105

AB A nucleic acid affinity matrix containing a short oligodeoxynucleotide ligand has been prepared as an example of a material which can be used for

the rapid and effective isolation of sequence specific DNA binding proteins. Two complementary oligodeoxynucleotides have been employed, one of which contains a small 5'-spacer arm with a **terminal thiol** group. Using this **terminal thiol** group, the ligand can be covalently coupled to Tresyl-activated Sepharose 4B or Epoxy-activated Sepharose 6B via a thioether linkage. This approach allows

the specific attachment of the nucleic acid ligand via its 5'-terminus to the insoluble matrix. The double stranded affinity material was obtained by annealing of the complementary DNA fragment. As an example, we have used an eicosomer affinity column containing the sequence d(GAATTC) for the isolation of the Eco RI restriction endonuclease. Using a single column, the enzyme could be isolated by eluting the column with a single step or multistep gradient of increasing salt concentration. The enzyme was purified to 75%-85% homogeneity with yields of 0.1 mg to 0.2 mg from 0.5 g of cell paste.

NMR  
5:305-532  
S Cleve 238 1401-1403

Synthesis of oligonucleotides containing

2'-deoxy-6-thioguanosine at a predetermined site.

AUTHOR: Christopherson M S; Broom A D  
CORPORATE SOURCE: Department of Medicinal Chemistry, College of Pharmacy,  
University of Utah, Salt Lake City 84112.  
CONTRACT NUMBER: 5 P30 CA 42014 (NCI)  
5 R01 A127692  
SOURCE: NUCLEIC ACIDS RESEARCH, (1991 Oct 25) 19 (20) 5719-24.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199112  
ENTRY DATE: Entered STN: 19920124  
Last Updated on STN: 19920124  
Entered Medline: 19911202

AB A new approach has been devised for the synthesis of oligonucleotides containing 2'-deoxy-6-thioguanosine [d(s6G)]. The synthesis of oligonucleotides containing d(s6G) requires special protection and deprotection strategies to prevent the thione functionality from undergoing oxidation and hydrolysis. Previous attempted syntheses have neglected to address this problem. By using the cyanoethyl protecting group for the thione and phenoxyacetyl for the exocyclic amino group, it was possible to deprotect oligonucleotides with a mixture of sodium hydroxide and sodium hydrogen sulfide without any significant conversion of d(s6G) to deoxyguanosine. Application of this strategy will allow investigation of the structural as well as biological activity of d(s6G)-containing oligonucleotides.

Immobilization of polynucleotides on magnetic particles.

Factors influencing hybridization efficiency.

AUTHOR: Day P J; Flora P S; Fox J E; Walker M R

CORPORATE SOURCE: University of Birmingham, Department of Clinical Chemistry,

Edgbaston, U.K.

SOURCE: BIOCHEMICAL JOURNAL, (1991 Sep 15) 278 ( Pt 3) 735-40.

Journal code: 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199110

ENTRY DATE: Entered STN: 19911108

Last Updated on STN: 19911108

Entered Medline: 19911023

AB Immobilization of oligonucleotides containing 5'-**terminal thiol** groups on thiol-terminated paramagnetic Biomag beads via disulphide bond formation was investigated. Oligonucleotides are demonstrated to couple at high yields, the linkage is stable at 90 degrees

C and is reversible, and the immobilized oligonucleotide is available for complementary, but not non-complementary, hybridization. Specific hybridization capacity per micrograms of immobilized oligonucleotide exceeds that achieved with other forms of immobilization chemistries employing random attachment and/or specific end attachment of the oligonucleotide to the solid support. Adsorption of DNA on the surface of the beads was decreased by incubation in 0.2% SDS; other non-specific blocking agents had no effect. Brief heating of the beads possessing immobilized oligonucleotides at 90 degrees C before hybridization increased the amount of specific hybridization dependent upon the inclusion of poly(dT) spacer sequences 5' to the immobilized oligonucleotide and 3' to the thiol group. Increasing lengths of spacers [up to a poly(dT16) spacer] linearly increased hybridization of complementary sequences.



FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 12:54:43 ON 18 MAR 2003

L1 14 S CHRISTOPHERSON M?/AU AND BROOM A?/AU  
L2 0 S THIOL? AND L1  
L3 7 DUP REM L1 (7 DUPLICATES REMOVED)  
L4 167 S TERMINAL THIOL  
L5 7 S L4 AND (BASE OR PYRIMIDINE OR PURINE)  
L6 6 S L4 AND NUCLEOTIDE  
L7 6 DUP REM L6 (0 DUPLICATES REMOVED)

=>

**WEST**☐ **Generate Collection** **Print**

L9: Entry 7 of 250

File: PGPB

Jan 30, 2003

DOCUMENT-IDENTIFIER: US 20030022227 A1

TITLE: Hybridization substrate, method of manufacturing same, and method of use for same

Summary of Invention Paragraph (22):

[0020] [11] A method of manufacturing the substrate of claim 6 in which DNA strands having a double-strand portion and a single-strand portion, with a thiol group being present on the terminal of said double-strand portion, and DNA strands comprised of only a double-strand portion on the terminal of which a thiol group is present are contacted with the metal surface of a metal substrate or substrate having a metal coating to immobilize said DNA strands on said metal surface.

Summary of Invention Paragraph (24):

[0022] [13] A method of manufacturing the substrate of claim 7 in which DNA strands having a double-strand portion and a single-strand portion, with a thiol group being present on the terminal of said double-strand portion, and DNA strands comprised of only a double-strand portion on the terminal of which a thiol group is present are contacted with the surface of a glass substrate or silicon substrate that has been surface treated with a hetero bifunctional crosslinking agent to immobilize said DNA strands on said surface.

Detail Description Paragraph (15):

[0049] The substrate of the present invention can be manufactured, for example, in the case of a metal substrate or a substrate having a metal coating, by contacting DNA strands having a double-strand portion and a single-strand portion with a thiol group present on the terminal of the double-strand portion, with the metal surface of the substrate to immobilize the DNA strands on the metal surface. As stated above, double-strand DNA having a double-strand portion and a single-strand portion consists of two single strands of different length, where the shorter strand of single-strand DNA has a nucleotide sequence complementing the nucleotide sequence of the longer strand of single-strand DNA from one of the ends thereof, and is manufactured by hybridizing the two single strands. For example, a thiol group is connected at the 5' end of a short single strand of DNA and this sequence of short single-strand DNA is hybridized with a long single strand of DNA having a complementary sequence on its 3' end to obtain a DNA strand having a double-strand portion and a single-strand portion with the double-strand portion having a terminal thiol group. A thiol group may be incorporated at the 5' terminal of the single-strand DNA by a known C6 synthesis method (for example, see Chemistry and Biology Experiments line 22, Tamba, Mineo, "DNA Chemical Synthesis", pp. 38-43, Hirokawa Shoten).

Detail Description Paragraph (18):

[0052] A mixture of a prescribed ratio of DNA strands having a double-strand portion, a single-strand portion, and a thiol group on the terminal of the double-strand portion to DNA strands having only a double-strand portion comprising a terminal thiol group can be contacted with the metal surface of a metal substrate or substrate having a metal coating in the same manner as set forth above to immobilize DNA strands having a double-strand portion and a single-strand portion and DNA strands having only a double-strand portion on the metal surface in a prescribed ratio.

Detail Description Paragraph (19):

[0053] When the substrate of the present invention is glass or silicon, for example, the substrate surface can be treated with a hetero bifunctional crosslinking agent,

1  
Mo  
Synthesizing RNA with a thiol at the 5'-terminus, for analysis and detection of RNA, comprises reacting a nucleoside with a chemical to form a thiol group at its 5'-terminus and treating with RNA polymerase to form a 5'-thiol-RNA molecule;

RNA synthesis for RNA array or RNA chip construction

AUTHOR: ZHANG B; CUI Z; ZHANG L  
PATENT ASSIGNEE: UNIV MASSACHUSETTS  
PATENT INFO: WO 2002044196 6 Jun 2002  
APPLICATION INFO: WO 2001-US44723 27 Nov 2001  
PRIORITY INFO: US 2000-253564 28 Nov 2000; US 2000-253564 28 Nov 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-618995 [66]  
AN 2003-01335 BIOTECHDS  
AB DERWENT ABSTRACT:

NOVELTY - Modifying the 5'-terminus of an RNA, by: (a) reacting a nucleoside with a chemical effective to yield a thiol group onto its 5'-terminus; (b) isolating the nucleoside comprising the 5'-terminus thiol molecule; and (c) subjecting the nucleoside comprising 5'-terminus thiol molecule to RNA polymerase, double-stranded DNA and NTPs under conditions suitable for an RNA polymerization reaction.

USE - The method is useful for modifying the 5'-terminus of an RNA molecule (claimed). The method finds applications in the analysis and detection of RNA, mapping RNA-protein interactions, in vitro selection

of

new catalytic RNAs, and gene array analysis. The thiol-modified RNA molecules are useful to bind a number of biological molecules, for e.g., proteins, peptides, enzymes, carbohydrates, nucleotides, oligonucleotides, DNA and detectable labels such as fluorophores,

biotin,

and dyes, and also to bind DNA containing a thiol reactive functional group (e.g., haloacetamides, maleimides, benzylic halides or bromomethylketones) to examine nucleic acid-nucleic acid interactions. They can also be used in the production of RNA bioarray chips.

EXAMPLE - Perchloric acid (4.1 ml) was added to a suspension of guanosine dissolved in 600 ml of acetone. Concentrated ammonium hydroxide

was added to the reaction mixture. The solid, 2',3'-isopropylideneguanosine (A) formed was then filtered out and reacted with methyltriphenoxy-phosphonium iodide to give

2',3'-isopropylidene-5'-deoxy-

5'-iodoguanosine (B). The crude product (B) was purified by flash chromatography and approximately 0.34 g (61.8 %) was obtained. (B) was deprotected by using approximately 50 % aqueous formic acid. Following deprotection, trisodium thiophosphate was added to the deprotected molecule which leads to the crude desired product, i.e., 5'-deoxy-5'-thioguanosine-5' -monophosphorothioate (GSMP). Once GSMP was formed, it was reacted with an RNA polymerase yielding a product that

was

subsequently treated with alkaline phosphatase to give 5'-HS-RNA, a 5'-terminal thiol RNA molecule. (57 pages)

L7 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:428924 CAPLUS

DOCUMENT NUMBER: 137:16574

TITLE: Enzymatic incorporation of sulfhydryl group into the 5'-terminus of RNA for 5'-sulfhydryl-modified RNA synthesis

INVENTOR(S): Zhang, Biliang; Cui, Zhiyong; Zhang, Lei

PATENT ASSIGNEE(S): University of Massachusetts, USA  
 SOURCE: PCT Int. Appl., 57 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002044196	A1	20020606	WO 2001-US44723	20011127
W: AU, CA, JP, MX, NZ, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
AU 2002017944	A5	20020611	AU 2002-17944	20011127
PRIORITY APPLN. INFO.:			US 2000-253564P	P 20001128
			WO 2001-US44723	W 20011127

AB The present invention pertains to methods for forming an RNA mol. that contains a 5'-terminal thiol group. There are at least two general protocols disclosed in the instant invention that leads to the formation of a 5'-thiol-RNA mol. One synthetic pathway leads to the formation of 5'-GSMP which is subsequently used as a substrate for an RNA polymerase forming a 5'-thiol-RNA mol. The method requires an addnl. step of dephosphorylation of 5'-GSMP-RNA to produce 5'-HS-G-RNA. Another synthetic pathway leads to the formation of 5'-HS-PEG-GMP which in turn is

also used as a substrate for an RNA polymerase. Generally, a nucleoside, such as guanosine, uridine, cytidine and adenosine can be used as the initial substrate in forming the modified RNA mol. The nucleoside is processed in such a manner as to render its 5' terminus receptive for receiving a thiol group. The thiol group can then be added to the nucleoside creating a modified 5'-thiol-mol. This nascent 5'-thiol mol. can then be subjected to transcription using an RNA polymerase, such as the T7 RNA polymerase, creating a 5'-thiol-RNA mol. In particular, the invention pertains to 5'-modified guanosines that can be used as initiators for T7 RNA polymerase, to directly incorporate a free thiol to 5'-termini of RNA by in vitro transcription. In one embodiment, the initiator is O-[[omega.-sulfhydrylbis(ethylene glycol)]-O-(5'-guanosine) monophosphate (5'-HS-PEG2-GMP). In another embodiment, the initiator is O-[[omega.-sulfhydryltetra(ethylene glycol)]-O-(5'-guanosine) monophosphate (5'-HS-PEG4-GMP). These initiators introduce a free thiol into 5'-end of RNA, and also provide a flexible PEG linker between HS group and RNA, which may be important for bioconjugation of mols. The detailed synthesis of 5'-deoxy-5'-thioguanosine-5'-monophosphorothioate, O-[[omega.-sulfhydrylbis(ethylene glycol)]-O-(5'-guanosine) monophosphate, and O-[[omega.-sulfhydryltetra(ethylene glycol)]-O-(5'-guanosine) monophosphate is described herein. The detailed syntheses of the sulfhydryl-modified guanosine monophosphates 5'-deoxy-5'-thioguanosine-5'-monophosphorothioate (GSMP), O-[[omega.-sulfhydryl-tetra(ethylene glycol)]-O-(5'-guanosine) monophosphate (5'-HS-PEG4-GMP), and O-[[omega.-sulfhydryl-di(ethylene glycol)]-O-(5'-guanosine) monophosphate (5'-HS-PEG2-GMP) are reported.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L7 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 ACCESSION NUMBER: 2001-08160 BIOTECHDS

TITLE: New **nucleotide**, useful for the covalent tagging of RNA molecules with gold clusters, comprises a sugar group, a phosphodiester, a pyrimidine and a **terminal thiol group**;  
structural characterization of protein-RNA complexes and DNA probe

AUTHOR: Sperling J; Medalia O  
PATENT ASSIGNEE: Yeda-Res.Develop  
LOCATION: Rehovot, Israel.  
PATENT INFO: WO 2001020017 22 Mar 2001  
APPLICATION INFO: WO 2000-IL564 13 Sep 2000  
PRIORITY INFO: IL 1999-131889 14 Sep 1999  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2001-244817 [25]

AN 2001-08160 BIOTECHDS

AB A **nucleotide** (I) is claimed. (I) has a natural sugar group or its sugar analog, a natural phosphodiester or any internucleosidyl linkage, a natural pyrimidine or purine base or their base analogs, and

a **terminal thiol group** at a side chain, which is covalently linked to the pyrimidine or purine base or their base analogs.

Also claimed are: a nucleic acid (II); and labeling a nucleic acid molecule at random locations with a metal comprising attaching metal atoms to the free thiol groups of (II), where the attachment of gold clusters at random locations in nucleic acid involves preparation of precursor (NTPs) and (rNTPs), incorporation of these precursor molecules into DNA or RNA in reaction catalyzed by DNA-polymerase (EC-2.7.7.7) or RNA-polymerase (EC-2.7.7.6), and attachment of gold clusters to the free thiol group. The nucleotides are useful for the covalent tagging of RNA molecules with gold clusters, enabling their direct visualization by microscopical methods. This is useful for structural characterization

of protein-RNA complexes and in microelectronic devices. The metal-tagged nucleic acids are also useful as DNA probes for macromolecular assemblies. (33pp)

L7 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:208441 CAPLUS

DOCUMENT NUMBER: 134:222979

TITLE: Preparation of metal cluster containing nucleotides and nucleic acids, and intermediates therefor

INVENTOR(S): Sperling, Joseph; Medalia, Ohad

PATENT ASSIGNEE(S): Yeda Research and Development Co. Ltd., Israel

SOURCE: PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001020017	A2	20010322	WO 2000-IL564	20000913
WO 2001020017	A3	20011004		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,

SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,  
 YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,  
 DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,  
 CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  
 AU 2000073095 A5 20010417 AU 2000-73095 20000913  
 EP 1244681 A2 20021002 EP 2000-960948 20000913  
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
 IE, SI, LT, LV, FI, RO, MK, CY, AL  
 PRIORITY APPLN. INFO.: IL 1999-131889 A 19990914  
 WO 2000-IL564 W 20000913  
 AB Nucleotides including a sugar moiety, a pyrimidine or purine base and a  
**terminal thiol** group at a side chain covalently linked  
 to the pyrimidine or purine base of the **nucleotide**, and  
 optionally further including a metal cluster covalently linked through  
 the **terminal thiol** group at said side chain to the  
 pyrimidine or purine base of the **nucleotide**, and nucleic acids  
 incorporating same. The attachment of gold-clusters at random locations  
 in a nucleic acid mol., comprising: (i) prepn. of precursor  
 deoxyribonucleoside triphosphates (NTP) and ribonucleoside triphosphates  
 (rNTP) whose heterocyclic ring contains substituents with a  
**terminal thiol** group (NTP-SH and rNTP-SH, resp.); (ii)  
 incorporation of these precursor mols. into DNA or RNA in reactions  
 catalyzed by DNA polymerase or RNA polymerase, resp.; and (iii)  
 attachment of gold-clusters to the free thiol groups, either by reacting with a com.  
 available maleimido deriv. of the cluster, or by reacting with colloidal  
 gold of pre-detd. size.

L7 ANSWER 5 OF 6 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
 ACCESSION NUMBER: 95065400 EMBASE  
 DOCUMENT NUMBER: 1995065400  
 TITLE: Stabilization of double-stranded oligonucleotides using  
 backbone-linked disulfide bridges.  
 AUTHOR: Gao H.; Yang M.; Cook A.F.  
 CORPORATE SOURCE: PharmaGenics Inc., 4 Pearl Court, Allendale, NJ 07401,  
 United States  
 SOURCE: Nucleic Acids Research, (1995) 23/2 (285-292).  
 ISSN: 0305-1048 CODEN: NARHAD  
 COUNTRY: United Kingdom  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 022 Human Genetics  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English  
 AB A convenient, practical route to the synthesis of disulfide-bridged  
 oligonucleotides has been developed. Aliphatic linkers with  
**terminal thiol** groups have been attached to the  
 phosphodiester backbones of partially or fully complementary  
 oligonucleotide sequences and oxidized to yield covalently closed  
 oligonucleotides with disulfide bridges. This procedure has been used to  
 prepare a duplex with disulfide bridges at both ends and stem-loop  
 sequences with single disulfide bridges. Oxidation of a  
 self-complementary  
 duplex possessing **terminal thiol** groups produced both  
 hairpin and duplex structures with disulfide bridges, the relative  
 proportions of each being dependent upon the reaction conditions. These  
 bridged hairpin and duplex structures were shown to be interconvertible  
 by  
 reduction and re-oxidation. The melting profiles of disulfide-bridged

oligonucleotides were compared with the same sequences without bridges and with sequences possessing triethylene glycol bridges, and in all cases the introduction of disulfide bridges resulted in a considerable increase in thermal stability. EcoRI endonuclease was capable of cleaving a disulfide-bridged duplex possessing a recognition site for this enzyme, thus supporting a lack of distortion of the recognition site. The disulfide bridges could be cleaved using a large excess of DTT to regenerate the corresponding sulfhydryl compounds. A study of the serum stabilities of disulfide-bridged oligonucleotides showed that the bridged duplexes were much more stable than their unmodified counterparts, whereas the rate of degradation of the stem-loop structures was more dependent upon the size of the loop than the presence or absence of the disulfide bridge. In summary, we have described a novel methodology, employing commercially available reagents, for the stabilization of oligonucleotide duplexes or stem-loop structures by disulfide bridge formation.

L7 ANSWER 6 OF 6 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
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AB Immobilization of oligonucleotides containing 5'-**terminal thiol** groups on thiol-terminated paramagnetic Biomag beads via disulphide bond formation was investigated. Oligonucleotides are demonstrated to couple at high yields, the linkage is stable at 90.degree.C and is reversible, and the immobilized oligonucleotide is available for complementary, but not noncomplementary, hybridization. Specific hybridization capacity per .mu.g of immobilized oligonucleotide exceeds that achieved with other forms of immobilization chemistries employing random attachment and/or specific end attachment of the oligonucleotide to the solid support. Adsorption of DNA on the surface of the beads was decreased by incubation in 0.2% SDS; other non-specific blocking agents had no effect. Brief heating of the beads possessing immobilized oligonucleotides at 90.degree.C before hybridization increased the amount of specific hybridization dependent upon the inclusion of poly(dT) spacer sequences 5' to the immobilized oligonucleotide and 3' to the thiol group. Increasing lengths of spacers [up to a poly(dT16) spacer] linearly increased hybridization of complementary sequences.

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